REF IS-3400	IDS-iSYS Direct Renin	
Instructions for Use	*IN VITRO DIAGNOSTIC*	CE

Intended Use

For In Vitro Diagnostic Use

The IDS-iSYS Direct Renin assay is intended for the quantitative determination of Direct Renin in human EDTA plasma on the IDS-iSYS Multi-Discipline Automated System. Results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the assessment of Hypertension related syndromes.

Summary and Explanation

Renin is a proteolytic enzyme synthesised by the juxtaglomerular cells of the kidney. Renin plays an integral role in the Renin-Angiotensin-Aldosterone System (RAAS). The RAAS regulates blood pressure through blood volume, sodium and water homeostasis control and vasoconstriction. The biochemical action of renin is to cleave Angiotensinogen to Angiotensin I. Angiotensin I is immediately transformed to Angiotensin II by Angiotensin Converting Enzyme (ACE). Angiotensin II is a small potent molecule able to regulate blood pressure through vasoconstriction. Angiotensin II is the active hormone and is regulated by aldosterone level. An over active RAAS leads to vasoconstriction and retention of sodium and water which can lead to hypertension. Consequently, assessment of the RAAS is essential for diagnostic and therapeutic purposes.

1 in 3 people worldwide will develop hypertension regardless of age and gender. Hypertension can be classified as Primary (essential), in around 80% of hypertensive patients, and Secondary, in around the remaining 20% of hypertensive patients.

Plasma renin measurement is important in stratifying the diagnosis of the two most common forms of secondary hypertension which are Primary Aldosteronism and Renovascular Hypertension. Plasma renin evaluation can also help in selecting the most appropriate antihypertensive treatment

Method Description

The IDS-iSYS Direct Renin kit is based on chemiluminescence technology. Two monoclonal antibodies against renin are utilised. One is labelled with acridinium and the other with biotin.

Samples are incubated with both labelled antibodies along with buffer A and buffer B. Streptavidin coated magnetic particles are added and following a further incubation step, the particles are “captured” using a magnet. After a washing step and addition of trigger reagents, the light emitted by the acridinium label is directly proportional to the concentration of renin in the original sample.

Warnings and Precautions

The IDS-iSYS Direct Renin kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in these Instructions for Use (IFU). IDS Limited will not be held responsible for any loss or damage (except as required by statute), arising out of non-compliance with the instructions provided.

CAUTION: This kit contains material of animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Appropriate precautions and good laboratory practice must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Sodium Azide
Calibrators CAL A / CAL B, contain sodium azide as preservative >0.1% <1% (w/w)
Xn. Harmful: Calibrators contain sodium azide >0.1% <1% (w/w)

R22	Wear suitable protective clothing and gloves.
R52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S46	If swallowed, seek medical advice immediately and show this container or label.
S36/37	Wear suitable protective clothing and gloves.
S60	This material and/or its container must be disposed of as hazardous waste.

Various components of this kit contain sodium azide which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

Handling Precautions
Apart from the calibrators which are lyophilised, the reagents provided in the kit are ready to use. Refer to the calibrator section of the procedure for reconstitution methodology.
Before a new cartridge is loaded onto the system, the magnetic particle container requires mixing by the operator with a brisk rotation motion. This will resuspend the magnetic particles that have settled during shipment. It is very important to avoid any foam formation.

Shelf Life and Storage of Reagents
Prior to first use, store the cartridge and the calibrators in an **upright** position in the dark at 2 to 8 °C. Do not freeze the cartridge.

Reagent shelf life	Cartridge	Calibrators
Before opening at 2 - 8 °C	To the expiry date	
Cartridge, after opening at 2 - 8 °C	28 days	NA
On board the System **(*)**	28 days	8 hours

(*) Continuous on board stability.

Sample Collection and Storage

The assay should be performed using EDTA plasma samples. **To avoid cryo-activation, samples must be collected, centrifuged and stored at room temperature.**
Do NOT store plasma at 2-8°C as cryo-activation may occur.
Plasma samples must be stored deep frozen at -20°C
If samples are turbid and/or contain visible clots they should be centrifuged before testing.

Samples stored deep frozen at -20°C must be thawed quickly before testing. After thawing, if samples are turbid and/or contain visible clots they should be centrifuged before testing.

Avoid repeated freeze-thaw of samples.

Do not use severely lipaemic samples.

To minimise possible evaporation effects, samples, calibrators, and controls should be measured within 8 hours of being placed on the system.

Before assay, make sure that samples, calibrators and controls are at room temperature (20 - 25 °C).

It is recommended to follow the instructions of the tube manufacturer especially when processing samples in primary tubes.

The assay sample volume is 190 µL. The total sample volume required for a single determination is dependent on the dead volume of the sample tube is use (190 µL+ dead volume of sample tube).

Procedure

Materials Provided

Reagent Cartridge

MPT2

Magnetic particles coated with streptavidin in a phosphate buffer with sodium azide as a preservative (<0.1%). 1 bottle, 2.7mL.

CONJ

Anti-renin labelled with an acridinium ester derivative, in a phosphate buffer contain bovine protein with sodium azide as a preservative (<0.1%). 1 bottle, 4.4mL.

Ab-Biot

Anti-renin labelled with biotin in a phosphate buffer containing bovine protein with sodium azide as a preservative (<0.1%). 1 bottle, 7.25mL.

BUF A

Equine serum with sodium azide as a preservative (<0.1%). 1 bottle, 7.25mL.

BUF B

A phosphate buffer containing bovine and mouse proteins with sodium azide as a preservative (<0.1%). 1 bottle, 7.25mL.

Calibrators

CAL A
CAL B

An equine serum matrix containing renin with sodium azide as a preservative (<0.1%). 3 each of 2 concentration levels, 1mL

Mini CD

Contains IFU for IDS-iSYS reagents and CRY files.

Materials Required But Not Provided

IDS-iSYS Multi-Discipline Automated System:	IS-310400
IDS-iSYS Direct Renin Control Kit	IS-3430
IDS-iSYS Cuvettes Cube:	IS-CC100
IDS-iSYS System Liquid:	IS-CS100
IDS-iSYS Wash Solution:	IS-CW100
IDS-iSYS Triggers Set A and B:	IS-CT100
IDS-iSYS Cartridge Check System:	IS-6010

Disposable polypropylene 2 mL, 10.8 mm diameter, conical skirted base, screw cap micro tubes, and screw cap with O-ring (Sarstedt 72.609 and 65.716 or equivalent)
Precision Pipetting Devices
Distilled or Deionised water

Assay Procedure

Reagent Cartridge

The reagents provided in the cartridge are ready to use. The system automatically performs the mixing of magnetic particles to maintain homogeneity. Before a new cartridge is loaded on board the system, mix the magnetic particles container by brisk rotation motion. Avoid foam formation.

The barcode is read when the cartridge is loaded on the reagent tray. If the label cannot be read by the system barcode reader, a manual procedure exists to enter the barcode data (see the IDS-iSYS User Manual).

Load the cartridge on the reagent tray and wait for at least 40 minutes before starting the assay. If the cartridge is removed from the reagent tray, store the cartridge vertically at 2 - 8 °C in the dark.

Calibrators

The IDS-iSYS Direct Renin calibrators are lyophilised. Reconstitute immediately before use. Add 1.0 mL of distilled or deionised water to each bottle. Replace the stopper. Leave for 10 minutes to reconstitute. Invert calibrators gently before use. Pipette the entire contents of the calibrator bottle into disposable 2mL polypropylene conical skirted base, screw cap micro tubes and place on the system. Proceed according to the instructions of the IDS-iSYS User Manual. DO NOT return material to the calibrator vial.

System Calibration

The two Direct Renin calibrators are required to perform the adjustment of the master curve. The calibrators are supplied with the kit and calibrators from another lot must not be used. The reconstituted extracts kit controls MUST be run at the same time as the calibrators to perform a master curve adjustment.

All data required for the calibration of the cartridge batch can be found on the mini CD. Use calibrator levels A and B to adjust the master curve to the reagents on board the System. Check for the presence of a Direct Renin cartridge on the reagent tray and the availability of the cartridge master curve in the database. If the data for the lot of calibrators is not available on board the system, load the data using the mini CD provided with the calibrator.

Start the immunoassay calibration on the IDS-iSYS System according to the IDS-iSYS User Manual. The calibration is carried out in triplicate. RLU CVs of >7% will result in a failed calibration. One replicate may be removed to meet the calibration requirements. As stated above, please note that controls must also be run. Verify and approve the calibration according to the calibration status displayed in the calibration windows and discard the calibrator from the sample tray after use.

Calibration

The IDS-iSYS Direct Renin assay has been standardised against the WHO IS 68/356.

To obtain values expressed in pg/mL, it is necessary to divide the results with the IDS-iSYS kit by a factor of 1.67.

Calibration Frequency

A new calibration is required:

- Each time a new lot of cartridge is loaded onto the system.
- Each time a new lot of trigger or cuvettes is used.

- When the control values do not fall within the defined ranges.
- When the calibration interval of 14 days has expired.
- After System service.

Verification of the calibration is automatic and managed by the system.

Quality Control

To ensure validity of results at least three controls with varying levels of Renin should be measured. Controls should be tested at (or near) the beginning of every run containing patient samples and also during calibrations and according to local regulations. It is recommended that the controls be routinely run in duplicate. Laboratories should test controls at least once per shift.

Refer to the IDS-iSYS Direct Renin Control Set IFU for preparation and handling instructions.

Determination of Sample Renin levels

Process samples according to the IDS-iSYS User Manual.

Measurement Range (Reportable Range)

The reportable range of the assay is 1.8-550 µIU/mL. Any value that reads below 1.8 µIU/mL should be reported as "< 1.8 µIU/mL

Limitations of Use

1. As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
2. The performance characteristics of this assay have not been established in a paediatric population.
3. The following substances do not interfere in the IDS-iSYS Direct Renin Assay when the concentrations presented in the following table are below the stated threshold.

Potentially Interfering Agent	Threshold Concentration
Triglycerides	30mg/mL
Haemoglobin	250mg/dL
Bilirubin	0.2mg/mL
Red Blood Cells	0.4%
Biotin	25nM
Albumin	8.0 g/dL
Beta-2-Microglobulin	50µg/mL
Cathepsin B	0.1U/mL
Cathepsin D	0.5U/mL
Captopril	50µg/mL
Renitec (Enalapril maleate)	50µg/mL
Loxen Nicardipine HCl)	50µg/mL
Lasilix (Furosemide)	50µg/mL
Trypsin	1.5µg/mL
Plasmin	100µg/mL
Human anti-Mouse Antibody (HAMA)	30ng/mL
Rheumatoid Factor (Rf)	1000IU/mL

4. In patients receiving therapy with high biotin dose (i.e.>5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

Expected Values

Each laboratory should determine ranges for their local population. The following ranges were determined using the IDS-iSYS Direct Renin Assay and is provided for information only.

In order to determine the normal ranges for the IDS-iSYS Direct Renin assay, 244 Caucasian adult subjects, collected in the US, 18-65 years of age, with normal blood pressure (systolic/diastolic ≤120/80) and normal BMI (18-24.9) were analysed using the IDS-iSYS Direct Renin assay. Samples were taken between 7-10am after overnight fasting, upright (30 minutes standing or walking) and supine (lying down for at least 30 minutes)

	n	Mean	SD	Range
Upright	123	36.8 µIU/mL	22.2	5.3-99.1
Supine	121	24.0 µIU/mL	13.5	4.2-59.7

Performance Data

Representative performance data are shown. Results obtained at individual laboratories may vary.

Sensitivity

The limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" using 78 blanks and 118 low level samples.

Analytical Sensitivity	0.6 µIU/mL
LoD	1.8 µIU/mL
LoQ	5.0 µIU/mL

Precision

Precision was evaluated in accordance with a modified protocol based on CLSI EP-5A2, "Evaluation of Precision Performance of Quantitative Measurement Methods". Ten plasma controls were assayed using three lots of reagents in duplicate twice per day for 20 days on 3 instruments.

Concentration (µIU/mL)	n	Within-run		Total	
		SD	CV%	SD	CV%
16.3	80	1.1	7.0	1.6	9.6
17.3	80	1.1	6.6	1.9	11.2
18.0	80	0.7	4.1	1.6	8.8
53.9	80	1.7	3.1	5.0	9.3
130.5	80	7.8	6.0	10.2	7.8
184.0	80	14.2	7.7	15.8	8.6
199.8	80	6.1	3.1	14.0	7.0
302.6	80	8.0	2.6	20.0	6.6
418.2	80	13.4	3.2	30.9	7.4
444.5	80	27.6	6.2	37.7	8.5

Recovery

Average recovery in EDTA plasma was 103.9%

Linearity

Linearity was evaluated based on CLSI EP-6A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". Samples containing varying concentrations of Renin were assayed in duplicate. The resulting mean concentrations were compared to predicted concentrations. Samples were prepared by diluting a high patient sample with a low patient sample prior to assay.

14 sets of samples covering the range of the assay were measured, giving a total of 126 measured dilutions. Average Observed/Expected Direct Renin values were 94.9%, with an r-squared of 1.00 defined by linear regression analysis.

Method Comparison

The IDS-iSYS Direct Renin Assay was compared against a commercially available immunoassay for the quantitative determination of Renin, following CLSI EP-9A2, "Method Comparison and Bias Estimation Using Patient Samples". A total of 164 samples, selected to represent a wide range of Renin concentrations [2.0-385 µIU/mL], were assayed by each method.

Linear regression analysis was performed on the comparative data:
IDS-iSYS = 1.06 (x) - 0.22

(95% CI of the slope and intercept were 1.04 to 1.09, and -1.00 to +0.67 respectively); correlation coefficient (r-squared) = 0.98.

Specificity

Analyte	Cross-Reactivity
Renin	100%
Pro-Renin	≤2.8%

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